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54. Title of the invention:

Method of producing protein having factor VII activity

57. Abstract:

Problem: Novel method of producing protein having factor VIIa activity.

Solution: Method characterized in that mammalian cells are cultured into which DNA coding for protein having factor VIIa activity after activation has been inserted, and protein is obtained from this culture fluid and activated.

PATENT CLAIMS:

1. A method of producing protein having biological activity for blood coagulation mediated by factor VIIa comprising growing in an appropriate culture medium mammalian host cells containing a DNA construct containing a nucleotide sequence coding for a protein having the same or essentially the same biological activity for blood coagulation as factor VIIa having the following amino acid sequence:

[see extra sheet]

isolating the protein product encoded by said DNA construct and produced by said mammalian host cells, and activating said protein product and generating protein which has the same or substantially the same biological activity for blood coagulation as factor VIIa.

2. A method according to claim 1, including amplification of said DNA construct by cotransfection of said host cells with a gene coding for dihydrofolate reductase, wherein said appropriate medium contains methotrexate.

3. A method according to claim 1, wherein said protein product is activated by reacting it with a proteolytic enzyme selected from the group consisting of factor XIIa, factor IXa, kallikrein, factor Xa, and thrombin.

1	5	10	15
Glu Cys Lys	Glu Glu Gln Cys Ser Phe	Glu Glu Ala Arg	Glu Ile
20	25	30	
Phe Lys Asp	Ala Glu Arg Thr Lys Leu	Phe Trp Ile Ser Tyr	Ser
35	40	45	
Asp Gly Asp	Gln Cys Ala Ser Ser Pro	Cys Gln Asn Gly Gly	Ser
50	55	60	
Cys Lys Asp	Gln Leu Gln Ser Tyr Ile	Cys Phe Cys Leu Pro	Ala
65	70	75	
Phe Glu Gly	Arg Asn Cys Glu Thr His	Lys Asp Asp Gln Leu	Ile
80	85	90	
Cys Val Asn	Glu Asn Gly Gly Cys Glu	Gln Tyr Cys Ser Asp	His
95	100	105	
Thr Gly Thr	Lys Arg Ser Cys Arg Cys	His Glu Gly Tyr Ser	Leu
110	115	120	
Leu Ala Asp	Gly Val Ser Cys Thr Pro	Thr Val Glu Tyr Pro	Cys
125	130	135	
Gly Lys Ile	Pro Ile Leu Glu Lys Arg	Asn Ala Ser Lys Pro	Gln
140	145	150	
Gly Arg Ile	Val Gly Gly Lys Val Cys	Pro Lys Gly Glu Cys	Pro
155	160	165	
Trp Gln Val	Leu Leu Leu Val Asn Gly	Ala Gln Leu Cys Gly	Gly
170	175	180	
Thr Leu Ile	Asn Thr Ile Trp Val Val	Ser Ala Ala His Cys	Phe
185	190	195	
Asp Lys Ile	Lys Asn Trp Arg Asn Leu	Ile Ala Val Leu Gly	Glu
200	205	210	
His Asp Leu	Ser Glu His Asp Gly Asp	Glu Gln Ser Arg Arg	Val
215	220	225	
Ala Gln Val	Ile Ile Pro Ser Thr Tyr	Val Pro Gly Thr Thr	Asn
230	235	240	
His Asp Ile	Ala Leu Leu Arg Leu His	Gln Pro Val Val Leu	Thr
245	250	255	
Asp His Val	Val Pro Leu Cys Leu Pro	Glu Arg Thr Phe Ser	Glu
260	265	270	
Arg Thr Leu	Ala Phe Val Arg Phe Ser	Leu Val Ser Gly Trp	Gly
275	280	285	
Gln Leu Leu	Asp Arg Gly Ala Thr Ala	Leu Glu Leu Met Val	Leu
290	295	300	
Asn Val Pro	Arg Leu Met Thr Gln Asp	Cys Leu Gln Gln Ser	Arg
305	310	315	
Lys Val Gly	Asp Ser Pro Asn Ile Thr	Glu Tyr Met Phe Cys	Ala
320	325	330	
Gly Tyr Ser	Asp Gly Ser Lys Asp Ser	Cys Lys Gly Asp Ser	Gly
335	340	345	
Gly Pro His	Ala Thr His Tyr Arg Gly	Thr Trp Tyr Leu Thr	Gly
350	355	360	
Ile Val Ser	Trp Gly Gln Gly Cys Ala	Thr Val Gly His Phe	Gly
365	370	375	
Val Tyr Thr	Arg Val Ser Gln Tyr Ile	Glu Trp Leu Gln Lys	Leu
380	385	390	
Met Arg Ser	Glu Pro Arg Pro Gly Val	Leu Leu Arg Ala Pro	Phe
395	400	405	

Pro

Figure 5 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 6 illustrates the Factor VII cDNA sequence of λ VII 2463.
Figure 7 illustrates the Factor VII cDNA sequence of λ VII 2463.